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Effect of Leptin Replacement on Brain Structure in Genetically Leptin-Deficient Adults

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The hormone leptin profoundly affects body weight and metabolism. Three human adults (two women, 35 and 40 yr old; one man, age 27) have been identified with a recessive mutation in the *ob* gene, which is homologous to the mutation in *ob/ob* mice, and produces leptin deficiency and morbid obesity. Because leptin replacement increases brain weight and changes brain protein and DNA content in *ob/ob* mice, we hypothesized that analogous treatment of leptin-deficient humans would alter brain tissue composition. Volumetric T1-weighted magnetic resonance images of the brain were acquired before and at 6 and 18 months after initiation of replacement therapy (daily sc injections of recombinant methionyl human leptin), which produced dramatic loss in body weight. We used voxel-based morphometry to test for increased gray matter tissue concentration after initiation of leptin replacement and detected increases at 6 months in the anterior cingulate gyrus, the inferior parietal lobule, and the cerebellum. These increases were maintained for over 18 months, with identical stereotaxic coordinates of the maxima for the effects. Our findings suggest that leptin can have sustained effects on tissue composition in the human brain and broaden the potential spectrum of leptin's influence beyond feeding behavior and endocrine function. (*J Clin Endocrinol Metab* 90: 2851–2854, 2005)

THE HORMONE LEPTIN, encoded by the *ob* gene, has strong influence on body weight and metabolism. These effects are thought to be mediated by neural circuits activated when the hormone, released from adipose tissue, interacts with leptin receptors in the brain. Although the study of leptin has advanced our understanding of the mechanisms that influence obesity (1), information on how the hormone affects brain function and structure is not well documented. Recent evidence suggests that leptin can have effects on neuronal plasticity within the hypothalamus of the *ob/ob* mouse (2, 3).

We studied the effects of leptin replacement in the brains of three genetically leptin-deficient adults, the only ones identified so far in the world. Two women and one man, from a highly consanguineous extended Turkish pedigree, were identified with a recessive *ob* gene mutation (cysteine to threonine in codon 105), homologous to the mutation in *ob/ob* mice. The resultant leptin deficiency produces multiple hypothalamic abnormalities, punctuated by morbid obesity. Leptin replacement therapy for the three adults consisted of daily sc injections of recombinant methionyl human leptin over the 18 months of study reported here (4).

Structural magnetic resonance (MR) images of the brain were acquired on the three leptin-deficient adults before the initiation of therapy and after 6 and 18 months of leptin replacement. Voxel-based morphometry was used to evaluate possible changes in tissue composition (gray matter concentration) in MR images on a voxel-wise basis (5). In voxel-based morphometry, the concentration of a particular class of tissue (*e.g.* gray matter) within each voxel is inferred from a weighted average of the intensity values of the neighboring voxels contained within the applied smoothing kernel. Because the range of signal intensities in a brain region on a T1-weighted MR image reflect the relative components of gray matter or white matter, the weighted average intensity in a voxel reflects the tissue concentration of that voxel in the segmented gray matter or white matter image.

Because exogenous leptin administration in *ob/ob* mice increases brain weight and also increases brain protein and DNA content (6, 7), we hypothesized that analogous treatment of leptin-deficient humans would alter brain tissue composition. Therefore, we tested for increased gray matter tissue concentration from pretreatment levels at 6 and 18 months of leptin replacement therapy.

Subjects and Methods

Subjects

The three adults (two women, 35 and 40 yr old; and one 27-yr-old man) were recruited in Turkey, and after giving written informed consent, were admitted to the General Clinical Research Center at the University of California Los Angeles for study. There was a baseline adjustment period of 3 months after the subjects arrived in Los Angeles before initiation of leptin replacement therapy. They were allowed to eat *ad libitum* during their stay, and their level of physical activity was not restricted.

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Abbreviation: MR, Magnetic resonance.

Leptin replacement therapy

A detailed description of the treatment procedure has recently been published (4). The subjects received daily sc injections (each evening between 1800 and 2000 h) of recombinant methionyl human leptin (provided by Amgen, Inc.) at low physiological doses, in the range of 0.01–0.04 mg/kg, for the duration of the study. The dose was recalculated as subjects lost weight, and reduced when normal body mass index was achieved, to avoid excessive weight loss.

MR imaging

Structural MR images were acquired on a Philips Intera 1.5 Tesla scanner at baseline (*i.e.* before initiation of leptin replacement) and after 6 and 18 months of daily treatment. The parameters for the three-dimensional T1-weighted spoiled gradient-recalled acquisition scan were as follows: TE/TR = 4/24 msec, NEX = 1, flip angle = 35°, field of view = 24 cm, and matrix size = 256^2, with 124 sagittal slices of 1.22 mm thickness through the brain.

Voxel-based morphometry

Analysis of the structural MR images was performed using statistical parametric mapping software (SPM99; developed at the Wellcome Department of Cognitive Neurology, University College London). The images were spatially normalized by linear and nonlinear transformations into the standard stereotaxic coordinate space developed at the Montreal Neurological Institute (*i.e.* MNI coordinate space). The normalized MR images were resampled to an isotropic voxel size of 2 mm. The images were segmented, using a modified mixture cluster algorithm with bias correction for magnetic field inhomogeneity, to produce separate images of gray matter and white matter. The gray matter images were then spatially normalized to the MNI gray matter template images provided in SPM99, to reduce the probability of voxel misclassification. Images from each testing session (baseline, 6 months, and 18 months) were spatially transformed to MNI space independently. The segmented images were finally smoothed with a 12-mm^3 isotropic Gaussian kernel to conform the images to the assumptions of random fields theory for statistical analysis (8).

Statistical analysis of MR images

Using the general linear model approach implemented in SPM99, relative differences (changes from baseline MR measurement) in average signal intensity (presumably reflecting changes in tissue composition) were determined within the segmented gray matter volume. All images were proportionally scaled to account for global differences. One-sided contrasts were performed to evaluate increased gray matter tissue concentration (inferred from relative signal intensity) at 6 and at 18 months *vs.* baseline. The voxel-wise threshold was set at $P < 0.0001$, and clusters of contiguous voxels that passed this threshold were considered significant at $P < 0.001$ corrected for multiple comparisons within the entire gray matter volume.

Results

The endocrine and metabolic effects of leptin replacement in the three adults over an 18-month period have recently been published (4). The treatment produced dramatic body weight loss in all three adults, corresponding to a reduction of between 44 and 53% of baseline weight, with a preferential loss of fat. The subjects also showed improvements in disordered neuroendocrine function (4), analogous to those observed in *ob/ob* mice.

At 6 and 18 months after initiation of replacement therapy, increases in relative gray matter tissue concentration were detected in clusters of contiguous voxels within the frontal cortex, primarily in the left anterior cingulate gyrus, and in the left inferior parietal lobule and the left cerebellum (Table 1 and Fig. 1). No significant effect occurred in the hypothalamic region. The voxels of peak or maximum effect within significant clusters in the left anterior cingulate gyrus ($x = -6$, $y = 32$, $z = 22$), left inferior parietal lobule ($x = -46$, $y = -46$, $z = 48$), and left cerebellum ($x = -10$, $y = -46$, $z = -46$) were at identical stereotaxic coordinates in scans acquired nearly a year apart (*i.e.* at 6 and 18 months after initiation of leptin replacement). Although the significant clusters were smaller (*i.e.* fewer voxels) after 18 (*vs.* 6) months of leptin replacement, the effect in these three regions was sustained (Table 1). All three subjects showed the same direction of effect over the three measurements, *i.e.* an increase from baseline values in gray matter concentration in the significant voxel clusters.

Discussion

Our results indicate increased gray matter tissue concentration in the brains of the three genetically leptin-deficient adults after leptin replacement. We tested for increased gray matter concentration and detected clusters in the anterior

TABLE 1. Increases in gray matter tissue concentration after leptin replacement

Regions	Cluster (no. of voxels)	Cluster-level *P* value (corrected)	z score	MNI coordinates (at maxima)		
				x	*y*	*z*
Increases at 6 months from baseline:						
R superior frontal	7	0.001	4.34	12	60	−4
L anterior cingulate	**56**	**0.0001**	**4.82**	**−6**	**32**	**22**
L cingulate	9	0.0001	3.90	−2	18	34
L inferior frontal	23	0.0001	4.54	−52	6	18
L inferior frontal	13	0.0001	4.81	−46	4	22
R medial frontal	9	0.0001	4.21	2	−10	62
L cingulate	14	0.0001	4.64	−4	−18	34
L cerebellum	**17**	**0.0001**	**5.48**	**−10**	**−46**	**−46**
L inferior parietal	**16**	**0.0001**	**4.38**	**−46**	**−46**	**48**
Increases at 18 months from baseline:						
L anterior cingulate	**33**	**0.0001**	**4.58**	**−6**	**32**	**22**
L cerebellum	**9**	**0.0001**	**5.40**	**10**	**−46**	**−46**
L inferior parietal	**12**	**0.0001**	**4.22**	**−46**	**−46**	**48**

The regions presented in *bold* exhibited sustained effects during leptin treatment (*i.e.*, they contained clusters with significant change from baseline or pretreatment values at both 6 and 18 months after initiating replacement therapy). Voxel-wise threshold was set at $P < 0.0001$ (uncorrected), and cluster-level significance was set at $P < 0.001$ (corrected). R, Right side of brain; L, left side.

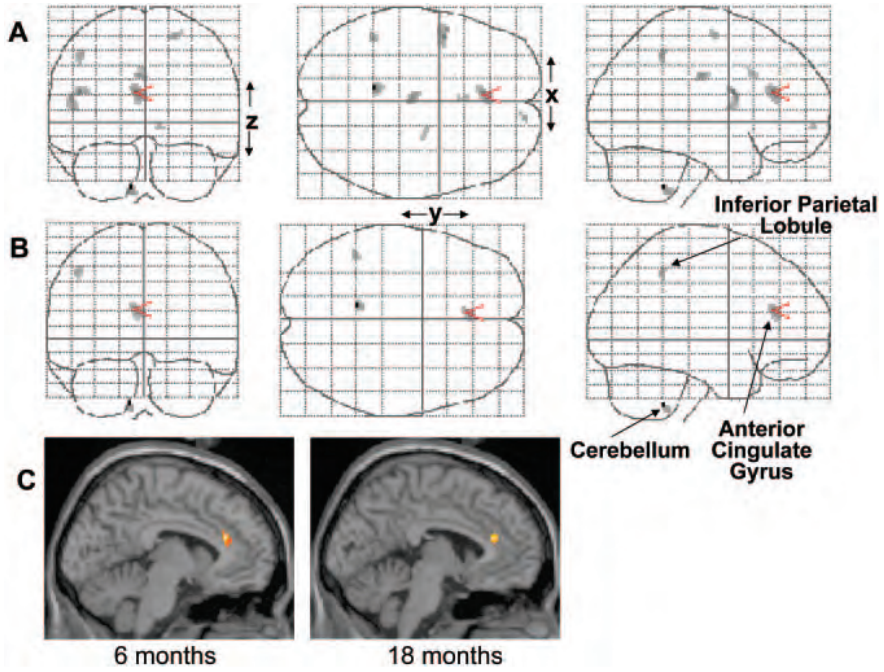

FIG. 1. Increased gray matter concentration at 6 (A) and 18 (B) months after initiating leptin replacement. Significant increases from baseline are displayed by maximum intensity projections (*i.e.* a glass brain) in coronal, transaxial, and sagittal orientations. The cursor indicates the voxel of maximum effect ($x = -6, y = 32, z = 22$) in the left anterior cingulate gyrus. The voxel-wise threshold was $P < 0.0001$, with clusters of contiguous voxels significant at $P < 0.001$, corrected for gray matter volume. C, Significant cluster in the anterior cingulate gyrus overlaid on a structural brain image. Stereotaxic coordinates were defined by the Montreal Neurological Institute.

cingulate gyrus, inferior parietal lobule, and cerebellum on the left side of the brain. The increases were sustained during continued treatment for over 18 months.

Recent findings suggest that leptin has plastic effects on neural connections between cell groups in the *ob/ob* mouse hypothalamus (2, 3). We did not detect any increase in tissue concentration in the hypothalamic region after exogenous leptin administration. Our data in only three leptin-deficient adults cannot rule out changes in the hypothalamus but do reveal significant effects on tissue composition in other regions of the brain. In this regard, it is notable that compared with wild-type mice, those that have the *ob/ob* mutation, and are leptin-deficient, have smaller volumes of many brain areas but not the hypothalamus (9). In addition, the effects of exogenous leptin administration in *ob/ob* mice, to ameliorate leptin deficiency, were not confined to the hypothalamic region but also affected global measures of brain structure, such as overall brain weight (6). Leptin receptors occur not only in the hypothalamus but also in other brain regions, including the cortex and cerebellum of the human brain (10). In the rat cingulate cortex, labeling of leptin receptors has been detected on neuronal cell bodies (11). Thus, our observations of regional increases in gray matter concentration are consistent with central effects of leptin at leptin receptors.

It is not known to what extent the increases in gray matter concentration reflect local interactions of the hormone with leptin receptors in the brain. Because leptin acts on neurons that coexpress neuropeptide Y, agouti-related protein, proopiomelanocortin, and cocaine- and amphetamine-related transcript (1), our findings could be secondary to the effects of exogenous leptin on one or more of these central-signaling molecules. The localized increases in gray matter concentration also may be secondary to peripheral effects of leptin or other circulating regulatory factors.

The observed effects may reflect increases in neuronal cell volume, reductions in focal myelination, or changes in the neuropil (*e.g.* dendritic processes). Voxel-based morphometry cannot provide information about the microstructure of a brain region or cytoarchitectonic details. The method can detect a change or a difference in voxel intensity (presumably reflecting tissue concentration), but the mechanism for that effect cannot be determined.

Limitations of the present study need to be recognized. Long-term obesity, as reflected by body mass index, has recently been associated with cerebral atrophy (loss of temporal lobe volume) measured by computed tomography in women (12). Thus, to further establish the validity of the present observations, it will be necessary to include a comparison group of non-leptin-deficient but weight-matched individuals. To our knowledge, voxel-based morphometry has not been used previously to access longitudinal changes in brain tissue composition. However, the identical location of stereotaxic coordinates for the maximum or peak voxel in the significant clusters at 6 months and again at 18 months provides some support for the reliability of the measurement procedure.

The anterior cingulate gyrus, the cerebellum, and the inferior parietal lobule, where we observed increases in gray matter concentration, have all been implicated in neural circuits regulating hunger and satiation in functional imaging studies (13). In this regard, it is interesting that all three of the subjects exhibited dramatic body weight loss although no restrictions were placed on their feeding (4). Nonetheless, the present study can only suggest that leptin may have extrahypothalamic effects on gray matter tissue concentration in the human brain. Although only three genetically leptin-deficient adults could be studied for this preliminary report, it will be important for future studies to extend the present findings, possibly including studies of individuals discovered with other *ob* gene mutations.

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